B 26 (cont)

acquire said heparanase catalytic activity, said protein being characterized by being about 50 or about 65 kDa, and said protein being characterized by being capable of being purified with a purification procedure initiated with Heparin-Sepharose chromatography, followed by gel filtration and pooling of active column fractions, wherein a quantity of said protein after said purification correlates with heparanase activity in said pooled active column fractions.

## REMARKS

Reconsideration of the above-identified application in view of the amendments above and the remarks following is respectfully requested. Claims 14-65 are in this case. Claims 14-65 have been rejected. Claims 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, and 64 have now been amended. New claims 66-70 have been added. The specification was also amended on pages 44, 51 and 52. Applicant notes that the Examiner required that either amended drawings or amended text be included with the response; Applicant has chosen to make the necessary corrections by amending the text.

## OBJECTED CLAIMS

The Examiner has objected to claims 44-53 because of the use of the phrase "having a pair of glutamic acids participating". The Examiner has suggested that this phrase be replaced with "having a pair of glutamic acid residues participating". The suggestion of the Examiner has been adopted in this regard, and claims 44, 46, 48, 50 and 52 have been correspondingly amended.

## Rejections over 35 USC 112

The Examiner has rejected claims 14-33 and 44-65 over 35 USC 112, first paragraph, as containing subject matter that was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s) had possession of the claimed invention. The rejections of the Examiner are respectfully

traversed. As the Examiner has made two sets of rejections under this section, Applicant has addressed each set of rejections separately.

In a first set of rejections, the Examiner has rejected claims 14, 15, 24, 44, 45, 54 and 55, for including the limitation "wherein said preparation is free of non-heparanase polypeptides encoded by human nucleic acid sequences". The Examiner has rejected claims 16, 17, 26, 27, 46, 47, 56 and 57 for including the limitation "wherein said isolated protein is substantially devoid of glycosylation". The Examiner has rejected claims 18, 19, 28, 29, 48, 49, 58 and 59 for including the limitation "wherein the preparation is substantially free of CXC chemokine or PAI1". The Examiner has rejected claims 20, 21, 30, 31, 50, 51, 60 and 61 for including the limitation "wherein said isolated protein is characterized by insect cell derived sugar prosthetic groups". The Examiner has rejected claims 22, 23, 32, 33, 52, 53, 62 and 63 for including the limitation "wherein said isolated protein is characterized by non-human cell derived sugar prosthetic groups". Applicant respectfully traverses the rejections of the Examiner.

Applicant notes that according to the revised "Guidelines for the Examination of Patent Applications Under the 35 USC 112, paragraph 1, 'Written Description' Requirement", section IB ("New or Amended Claims"), the guidelines clearly state that "there is no *in haec verba* requirement", such that the wording of the new or amended claims does not need to be literally present in the specification. Instead, it is sufficient if the wording is "supported in the specification through express, implicit, or inherent disclosure".

The revised Guidelines also state, in the response to comment 24, that "case law hold[s] that a patent specification `need not teach, and *preferably omits*, what is well known in the art.' See Spectra-Physics, Inc. v. Coherent, Inc., 827 F.2d 1524, 1534, 3 USPQ2d 1737, 1743 (Fed. Cir. 1987); Hybritech Inc. v. Monoclonal Antibodies, Inc., 802 F.2d 1367, 1384, 231 USPQ 81, 94 (Fed. Cir. 1986). See also Atmel Corp. v. Information Storage Devices, Inc., 198 F.3d 1374, 1382, 53 USPQ2d 1225, 1231 (Fed. Cir. 1999)" (emphasis added).

Applicant respectfully traverses the rejections of the Examiner as follows. All of these limitations have clear support in the specification, for example with regard to the expression of recombinant heparanase protein in insect cells (see page 78 of the

specification). In an insect cell expression system, any resultant preparation will automatically be "free of non-heparanase polypeptides encoded by human nucleic acid sequences", because the only human gene introduced to such a system would be the heparanase gene. Therefore, the limitation is implicitly supported in the disclosure, and would be clearly recognizable by one of ordinary skill in the art. As noted in the revised Guidelines above, a patent specification *preferably omits* that which was known in the art at the time of filing. At the time of filing of the present application, it was clearly known that insect cells will only produce a human protein if a human gene encoding for that protein is introduced to the insect cells; indeed as described in greater detail below, inducing insect cells to produce the human protein from the human gene is non-trivial, and would not automatically be expected to work by one of ordinary skill in the art. However, once the production of the human protein by the insect cells was shown to occur, it would be clear to one of ordinary skill in the art that the preparation would not include any other human proteins, other than that for which the introduced gene encodes.

Similar arguments may be made for the remaining limitations. For example, in microbial or insect cell expression systems, proteins such as CXC chemokines and/or PAI1 do not exist, so a heparanase protein produced in one of these expression systems would not be contaminated by one of these proteins. On the other hand, these proteins are well known contaminants of heparanase purified from non-recombinant sources, such as constitutive heparanase expression in mammalian cells. For example, the CXC chemokine CTAP III is a protein that was reported to possess heparanase-like activity and was mistakenly identified as heparanase by Hoogewerf et al. (WO 95/04158 as cited by the Examiner (see rejections over 35 U.S.C. 102 below) and J Biol Chem 17;270(7):3268-77, (1995) and by Kosir et al. (J Surg Res. 67(1):98-105, 1997. CTAP III is a low molecular weight chemokine, which has no homology to heparanase. Three years ago it was declared and it is now accepted, as is recited below, that CTAPIII was erroneously thought to be heparanase.

In a recent paper the same group from Pharmacia and Upjohn, Inc. retracted their earlier statement regarding the heparanase activity of CTAPIII (Fairbank et al. J Biol Chem

274(42): 29587-29590, 1999) page 29590, right column, last paragraph of the discussion. They state that:

> Finally, an earlier report from this laboratory suggested that heparanase was a post-translationally modified form of a CXC chemokine, namely CTAPIII (7). We have not been able to confirm this observation, nor have others who have purified and characterized human heparanase

In this paragraph they refer to their previous paper, Hoogewerf et al. (J Biol Chem 1995 Feb 17;270(7):3268-77) as discussed above. PCT Application No. WO 95/04158 refers to these same findings.

In addition, Applicant notes that the Examiner has rejected the above claims for lack of clarity, for reciting the terms "CXC chemokine", "PAI1" or "prosthetic group". The rejections of the Examiner are respectfully traversed.

Applicant notes that the term "PAI1" is used in Fuks, as cited by the Examiner (see col 15, lines 18-21), as an acronym for "type 1 plasminogen activator inhibitor". Therefore, clearly the term "PAI1" was known in the art at the time of filing the present specification.

The term "CXC chemokine" refers to a well known contaminant of native heparanase (heparanase obtained from constitutive mammalian expression). The "CXC chemokine" is actually a group of chemokines which are well known in the art. The term is well accepted and is broadly used (see for example Brandt E, et al. The beta-thromboglobulins and platelet factor 4: blood platelet-derived CXC chemokines with divergent roles in early neutrophil regulation J Leukoc Biol 2000 Apr;67(4):471-8; Strieter Rmet al. .CXC chemokines in angiogenesis related to pulmonary fibrosis. Chest 2002 Dec;122(6 Suppl):298S-301S).

With regard to the term "prosthetic group", Applicant has chosen to replace it with "post-translational modifying group". Various groups are well known in the art to be added to proteins after the polypeptide has been produced. These modifying groups are well known in the art to vary by genus, such that insect cells or bacterial cells are well

known to produce different types of modifications than mammalian cells, for example (see for example Ailor E and Betenbaugh MJ, Modifying secretion and post-translational processing in insect cells. Curr Opin Biotechnol 1999 Apr;10(2):142-5). Therefore, both what is meant by this term, and also support for this term, are inherent in the specification of the present Application, in which insect cells are clearly taught as being suitable for producing the recombinant heparanase according to the present invention.

With regard to the second set of rejections, the Examiner has rejected claims 14-33 and 44-65 as being overly broad, given that the specification describes three amino acid sequences (SEQ ID NOs: 10, 14 or 44) and also nucleic acid sequences encoding for these amino acid sequences. The Examiner has stated that "there is no disclosure of any particular structure to function/activity relationship in the disclosed species".

While continuing to traverse the rejections of the Examiner, Applicant has chosen to amend claims 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, and 64 and to add new independent claim 66 and new dependent claim 67, in order to expedite the prosecution.

Each of these amended claims now recites that the heparanase protein includes "a polypeptide at least 60% homologous to at least one of SEQ ID NOs: 10, 14, or 44 or portions thereof". This amendment clearly overcomes the rejections of the Examiner, as a clear structural relationship is drawn between the species disclosed in the specification and the claimed polypeptide. In addition, a clear relationship is drawn between the function of the claimed polypeptide and that of the disclosed species. Applicant therefore feels that these amendments overcome the rejections of the Examiner in this regard. Support can be found throughout the specification, particularly on page 39, lines 16-20.

Dependent claim 67 includes the limitation that the polypeptide is characterized by being recombinant. Support can be found as above.

Applicant has also chosen to add new independent claims 68-70 to further distinctly point out and claim the present invention. Independent claim 68 recites a preparation comprising a recombinant protein, in which the protein includes a polypeptide encoded by a polynucleotide capable of inducing heparanase activity after transfection into a cell, in which the cell is characterized by lacking such heparanase

activity before transfection, with the resultant preparation being free of non-heparanase polypeptides encoded by human nucleic acid sequences, the polypeptide having a pair of glutamic acid residues participating in its active site. Applicant feels that this recitation includes a clear structure-function relationship, given the recitation of the polynucleotide that is capable of inducing heparanase activity. Support can be found throughout the specification, particularly on page 54, lines 16-21.

New independent claim 69 recites that the recombinant protein includes a polypeptide capable of being encoded by a polynucleotide capable of hybridizing to at least a portion of at least one of SEQ ID NOs: 9, 13, 42, or 43. Again, this provides a clear structure-function relationship with the disclosed species. Support can be found throughout the specification, particularly on page 64, lines 10-13; and page 80-2.

New independent claim 70 recites a recombinant protein being characterized by being about 50 or about 65 kDa, and also being characterized by being capable of being purified with a purification procedure initiated with Heparin-Sepharose chromatography, followed by gel filtration and pooling of active column fractions, wherein a quantity of the protein after the purification correlates with heparanase activity in the pooled active column fractions. Applicant feels that this recitation overcomes the rejections of the Examiner in that the structural limitations of size and also behavior with Heparin-Sepharose chromatography and gel filtration are all included. Furthermore, this claim recites a simple test which can be used to determine whether a recombinant protein falls within the boundaries of the claim, such that undue experimentation is not required. Support can be found throughout the specification, particularly on pages 90-91.

Applicant notes that the revised Guidelines state in footnote 42 that "examples of identifying characteristics include sequence, structure, binding affinity, binding specificity, molecular weight and length.... For example, unique cleavage by particular enzymes, isoelectric points of fragments, detailed restriction enzyme maps, a comparison of enzymatic activities or antibody cross-reactivity". Applicant feels that these recited limitations clearly fall within these categories of permissible identifying characteristics, which clearly distinguish the protein of Applicant and which clearly provide structure-function relationships.

**Rejections over 35 USC 102**

The Examiner has rejected claims 64 and 65 under 35 USC 102 as being anticipated by US Patent No. 5,362,641 to Fuks et al. (hereinafter "Fuks"). The rejections of the Examiner are respectfully traversed.

Fuks describes the purification of a protein which, as described below, results in the production of a mixture of proteins, of which PAI1 is a significant component, even after all of the described purification procedures of Fuks have been performed. In fact, later evidence has shown that the antibody raised by Fuks against heparanase is actually an anti-PAI1 antibody. Identification of this antibody as an anti PAI-1 antibody is discussed in U.S. Patent No. 5,968,822 (Application No. 08/922,170). Page 11, line 18 to page 12, line 2, recite in this respect that:

> Several years ago we prepared rabbit polyclonal antibodies directed against our partially purified preparation of human placenta heparanase. These antibodies, referred to in U.S. Pat. No. 5,362,641, were later found to be directed against plasminogen activator inhibitor type I (PAI-1) that was co-purified with the placental heparanase. These findings led to a modification of the original purification protocol to remove the PAI-1 contaminant.

Therefore, the antibody of Fuks cannot anticipate claims 64 and 65, as the antibody of claims 64 and 65 recognizes heparanase, while that of Fuks does not.

Applicant has also chosen to amend claim 64, as described in greater detail below with regard to the rejections over 35 USC 103 that cite Fuks.

The Examiner has also rejected claims 14-17, 22-23, 54-57 and 62-65 under 35 USC 102 as being anticipated by PCT Application No. WO 95/04158 to Hoogwerf et al. ("Hoogwerf"). The rejections of the Examiner are respectfully traversed.

The object of Hoogwerf is the provision of recombinant heparanase. However, the sequences taught by Hoogwerf are actually those of CTAP-III and related proteins, which are well known contaminants of native heparanase. As discussed above, a later article by Hoogwerf (J. Biol. Chem., 1995, vol 270, pp. 3268-3277), of which a copy is attached to the Affidavit accompanying this response, indicates that the sequences of

"heparanase", as well as antibodies raised against these sequences, were actually of CTAP-III, an unrelated protein.

Furthermore, even if Hoogwerf actually had determined heparanase sequences, the use of the present tense with regard to the description of the use of insect cells for expression of heparanase, combined that other parts of the application use the past tense for describing various experimental acts, clearly indicates that Hoogwerf had not performed any expression of heparanase with insect cells. The use of insect cells for expression human or other mammalian proteins, or indeed any non-insect protein, is non-trivial, and would not be expected to be operative by one of ordinary skill in the art, as described in greater detail below with regard to the rejections over Fuks, 35 USC 103.

In addition, Applicant has chosen to overcome the rejections of the Examiner by amending claims 14, 16, 22, 54, 56, 62, and 64, and by adding new claim 66. All of these amended claims now recite "a polypeptide at least 60% homologous to at least one of SEQ ID NOs: 10, 14, or 44 or portions thereof". As Hoogwerf does not recite any of these sequences, Applicant feels that this amendment overcomes the rejection of the Examiner in this regard.

With regard to new claims 68-70, Applicant notes that Hoogwerf does not teach or suggest a polynucleotide capable of inducing heparanase activity after transfection into a cell, the cell being characterized by lacking such heparanase activity before transfection, the preparation being free of non-heparanase polypeptides encoded by human nucleic acid sequences, the polypeptide having a pair of glutamic acid residues participating in its active site (claim 68); a polypeptide capable of being encoded by a polynucleotide capable of hybridizing to at least a portion of at least one of SEQ ID NOs: 9, 13, 42, or 43 (claim 69); or a protein being characterized by being about 50 or about 65 kDa, and also being characterized by being capable of being purified with a purification procedure initiated with Heparin-Sepharose chromatography, followed by gel filtration and pooling of active column fractions, wherein a quantity of the protein after said purification correlates with heparanase activity in the pooled active column fractions (claim 70).

**Rejections over 35 USC 103**

The Examiner has rejected claims 14-63 under 35 USC 103(a) as being anticipated by US Patent No. 5,362,641 to Fuks et al. (hereinafter "Fuks"). The rejections of the Examiner are respectfully traversed.

Fuks describes the purification of a protein which, as described below, results in the production of a mixture of proteins, of which PAI1 is a significant component, even after all of the described purification procedures of Fuks have been performed. The Examiner has asserted that the heparanase of Fuks is the same protein as that of the present invention. However, as described in greater detail below, the purification procedure of Fuks actually produced a mixture of proteins, of which a significant, or even dominant, component is PAI1. That the purification procedure of Fuks clearly produced a mixture of proteins, rather than a single pure protein, is further butressed by the later discovery that the "anti-heparanase antibody" described in Fuks is actually an anti-PAI1 antibody, as described above. Thus, the purification procedure of Fuks clearly produces a mixture of proteins which includes at least a significant amount of PAI1.

Fuks does not teach a protein or nucleotide sequence for heparanase. The Examiner has therefore stated that "one of ordinary skill in the art at the time of filing would have been motivated to isolate the cDNA that encodes the heparanase taught by Fuks et al. and express this cDNA in an expression system utilizing either bacteria, yeast or insect cells". Applicant specifically traverses this rejection of the Examiner by acknowledging that while motivation may exist for determining a protein or nucleic acid sequence, with regard to heparanase, motivation is not sufficient, as determining the protein/nucleic acid sequence of heparanase would require undue experimentation.

The standard for undue experimentation has been determined in a number of court cases. According to this standard, some experimentation may be required, but only of a routine nature. If routine experimentation would not be expected to produce the desired result, particularly because of the requirement for inventiveness in order to overcome problems with routine experiments, then experimentation becomes undue. If undue experimentation is required, such that inventiveness is necessary to produce an invention, then a reference cannot make a claimed invention obvious.

As described in greater detail below and in the attached Affidavit, Applicant found that following the procedure of Fuks led to the preparation of a mixture of proteins, of which heparanase was only a relatively small component overall. A significant number of experiments requiring inventiveness were then performed, any one of which would fall under the category of requiring undue, inventive experimentation. In fact, following the teachings of Fuks would only mark the beginning of a process which would not provide one of ordinary skill in the art with the claimed sequence, unless a number of inventive acts were performed. This apparent contradiction is due to the fact that regardless of whether purified proteins may, in general, be readily sequenced if a process for purifying a protein is known and available, heparanase itself represents a special, and specially difficult, case.

The problems begin when one of ordinary skill in the art follows the teachings of Fuks and obtains a mixture of proteins after the performance of Mono-S HPLC purification (col 16, lines 46-54 of Fuks). Fuks does not teach that use of such a purification step results in the production of a mixture of proteins, because such a step was never actually performed by the inventors of Fuks, as clearly demonstrated by the use of the present tense for this description of Mono-S HPLC purification. Use of the present tense is reserved for experiments which have been designed but not actually performed. Therefore, both the efficacy of such a purification process and the ability to obtain 8,000 fold purified heparanase are expectations and not demonstrated fact.

If one of ordinary skill in the art nevertheless chose to perform the taught purification process of Fuks through this HPLC purification step, such a person would be confronted with a mixture of proteins, as demonstrated by the attached Appendix A, provided by the inventors of the present Application, who did actually perform this step. The mixture would show heparanase activity, as in fact a portion of this mixture would include heparanase itself. However, it would also include other proteins, including PAI-1, a known contaminant of heparanase obtained through protein purification. Furthermore, one of ordinary skill in the art would know that all previous purification processes that had been touted as producing "pure" heparanase actually resulted in the purification of many different proteins, but not the enzyme heparanase. Appendix B

provides a listing of only a sample of some of the many different proteins which were thought to be "pure" heparanase.

As described in greater detail in the attached Affidavit, performing tryptic digestion and microsequencing on such a mixture of proteins would therefore result in multiple protein (amino acid) sequences being obtained. The inventors actually compared these sequences against the database of known proteins, and found that a number of such sequences did not match any known protein. These sequences are given in Appendix C, with some exemplary identified sequences from known proteins. Even at this stage, one of ordinary skill in the art would presumably be questioning the presence of multiple unidentified sequences.

Assuming that the correct cDNA clone was obtained, which is not an assured conclusion in any way, when a cDNA is cloned with a "complete" protein sequence according to the short sequence obtained through microsequencing, a polypeptide is obtained which has 543 amino acids and a calculated molecular weight of 61,178 daltons. Since heparanase is known to be glycosylated, the molecular weight is expected to be even higher . However, Fuks teaches that the *expected* molecular weight of heparanase is 50 kD (col 15, lines 19-23 and 50-55). Therefore, one of ordinary skill in the art would in fact believe that the wrong protein had been obtained, and would therefore be expected to cease further experiments with the "complete" protein sequence.

Even if one of ordinary skill in the art chose to persevere, against the experimental evidence, thereby showing inventiveness and again contradicting the standard, further difficulties would arise if such a person attempted to actually produce heparanase *in vitro* from the cloned gene, for example in order to examine the activity of the gene product. Such a person might be expected to produce heparanase by transfecting bacteria or yeast, for example. However, bacteria and yeast cannot produce active heparanase, as demonstrated in Appendix D. Attempting to transfect a mammalian cell line would result in the problem of being unable to distinguish heparanase activity produced by the product of the transfected heparanase gene, and that of native heparanase, since the most commonly used mammalian cell lines have basal endogenous heparanase activity, as seen for example in the cell lines 293 and CHO (Chinese hamster ovary cells) . One of ordinary skill in the art would thus assume that the heparanase

"gene" actually did not code for heparanase, as no activity would be observed from transfection into bacteria or yeast.

If one of ordinary skill in the art continued to persevere, having committed a number of inventive acts in an attempt to obtain the heparanase sequence (as described above), such a person would now be required to perform a truly inventive act in order to obtain active heparanase from the suspected gene, thereby yet again contradicting the standard. One of ordinary skill in the art would now need to transfect insect cells with the heparanase gene sequence, using the Baculovirus expression system. This act is truly inventive because insect cells are not regularly used for mammalian proteins, and would certainly not be the cell line of choice after yeast failed to produce an active heparanase protein product. Appendix E demonstrates why insect cells are not usually a cell line of choice for mammalian proteins, and also some of the typical uses for insect cells with the Baculovirus expression system for gene transfection.

As described in the present Application (page 78), the use of insect cells with the Baculovirus expression system results in the production of active heparanase gene product. However, there is a further potential source of confusion for one of ordinary skill in the art. The full length polypeptide for which the heparanase gene codes, which is of about 66 kDa, is actually a prepro-heparanase form of the protein, and has no heparanase activity. Instead, this form of the protein is cleavable to form a protein having heparanase activity.

A residual level of such activity can be detected due to the effect of non specific protease activity, which results in the activation of a minor fraction of the recombinant enzyme. The detection of heparanase activity was found to require several micrograms of recombinant enzyme, a quantity that is much higher than expected from a catalytic enzyme. This finding raised doubts about the functional identification of the cloned cDNA, and would clearly cause one of ordinary skill in the art to doubt whether the correct gene had been identified and cloned. Thus, if one of ordinary skill in the art had actually performed the necessary experiments, such a person would have assumed that the true heparanase gene had not been identified. Again, inventiveness would be required to overcome this assumption.

Although cleavage of the prepro-heparanase results in catalytic activity of the enzyme, attempts to obtain an actual heparanase protein through protein cleavage is likely to result in inactive protein. The activating protease is unknown and using non-specific proteases such as trypsin requires extensive studies in order to obtain an activated protein rather than a degraded protein. The pro-heparanase protein can be divided into three sections: an 8 kDa section, a 6 kDa section and the main 45 kDa section. In order to produce heparanase, the 6 kDa section is removed and the 8 kDa section joins to the 45 kDa section. Thus, one of ordinary skill in the art could not actually produce the 50 kDa protein through mere cleavage of the actual gene product, and would need to display a clearly inventive understanding of heparanase and of the gene itself in order to obtain the invention claimed in the present Application.

In any case, it should be noted that suspecting that the identified heparanase was a pre-pro form of the enzyme is in itself inventive, because one of ordinary skill in the art would have no basis for such a suspicion. Beyond this suspicion, however, one of ordinary skill in the art would need to understand how to ameliorate the problem. The present inventors were able to use insect cells with the Baculovirus system to overcome the twin problems of existing levels of heparanase activity in mammalian cells, and lack of activity after transfection of yeast cells. However, this solution is by no means obvious, for the reasons given above.

Applicant further notes that the present Application provides a full and complete description of all processes required to *reproduce* the present invention, since mere *reproduction* does not involve reproducing all of the inventive acts necessary to obtain the present invention. As described above, the present invention actually overcomes a number of significant barriers with inventive acts. Furthermore, each barrier represents several problems which must be overcome by a potential inventor, since the inventor would need to actually recognize the true nature of each barrier and the problem to be solved. For example, at the very beginning of the inventive process described above, the cloned cDNA would be expected to produce a polypeptide with a calculated molecular weight of 61,178 daltons, although Fuks teaches that the *expected* molecular weight of heparanase is 50 kDa. The true nature of the problem is therefore obscure. Does the gene product have a different molecular weight because it codes for the wrong protein? Is

the coded protein a pre or pro-heparanase? Or is some other factor responsible for this discrepancy? Indeed, does the gene product code for a protein that is even related to heparanase?

Applicant would also like to emphasize the impact of each prior stage on the expected interpretation of the next stage by one of ordinary skill in the art. Continuing with the example of the previous paragraph, the apparently contradictory protein molecular weights would have been evaluated by one of ordinary skill in the art in view of the microsequencing results. These results would have indicated that a mixture of proteins was present, contrary to the teachings of Fuks which emphasize a very high degree of purity. Thus, one of ordinary skill in the art would have understood the contradictory molecular weight as indicating that the wrong protein had been obtained.

Thus, one of ordinary skill in the art could not have obtained the heparanase sequence from the mixture of proteins produced according to the process of Fuks, without a large number of significant, inventive acts. It would not therefore be possible, even given motivation, to obtain the heparanase sequences of the present invention without being inventive, which directly contradicts the standard for obviousness. Applicant therefore feels that these arguments overcome the Examiner's rejections in this regard.

For the reasons given above, Applicant feels that claims 14-70 are in condition for allowance. A prompt Notice of Allowance is respectfully requested.

Respectfully submitted,

Sol Sheinbein

Registration No. 25,457

Date: 21 April 2003

MARKED-UP AMENDMENTS TO SPECIFICATION:

Amended page 44, paragraph from lines 14-20:

FIG. 1 presents nucleotide sequence and deduced amino acid sequence of *hpa* cDNA with regard to SEQ ID NO: 11. A single nucleotide difference at position 799 (A to T) between the EST (Expressed Sequence Tag) and the PCR amplified cDNA (reverse transcribed RNA) and the resulting amino acid substitution (Tyr to Phe) are indicated above and below the substituted unit, respectively. Cysteine residues and the poly adenylation consensus sequence are underlined. The asterisk denotes the stop codon TGA.

Amended page 51, paragraph from lines 1-12:

FIG. 16 presents the nucleotide sequence of the genomic region of the *hpa* gene with regard to SEQ ID NO: 42. Exon sequences appear in upper case and intron sequences in lower case. The deduced amino acid sequence of the exons is printed below the nucleotide sequence. Two predicted transcription start sites are shown in bold.

FIG. 17 presents an alignment of the amino acid sequences of human heparanase, mouse and partial sequences of rat homologues with regard to SEQ ID NOs: 10, 44 and 45. The human and the mouse sequences were determined by sequence analysis of the isolated cDNAs. The rat sequence is derived from two different EST clones, which represent two different regions (5' and 3') of the rat *hpa* cDNA. The human sequence and the amino acids in the mouse and rat homologues, which are identical to the human sequence, appear in bold.

Amendment of page 52, lines 1-5:

FIG. 19 demonstrates the secondary structure prediction for heparanase (SEQ ID NO:10) performed using the PHD server – Profile network Prediction Heidelberg. H – helix, E – extended (beta strand), The glutamic acid predicted as the proton donor is marked by asterisk and the possible nucleophiles are underlined.

Marked-up Claims:

14. (Amended) A preparation comprising a protein having heparanase (endo- β -D-glucuronidase) catalytic activity or being cleavable so as to acquire said heparanase catalytic activity, said protein including a polypeptide at least 60% homologous to at least one of SEQ ID NOs: 10, 14, or 44 or portions thereof, the preparation being free of non-heparanase polypeptides encoded by human nucleic acid sequences.

16. (Amended) An isolated protein having heparanase (endo- β -D-glucuronidase) catalytic activity or being cleavable so as to acquire said heparanase catalytic activity, said protein including a polypeptide at least 60% homologous to at least one of SEQ ID NOs: 10, 14, or 44 or portions thereof, said isolated protein being substantially devoid of glycosilation.

18. (Amended) A preparation comprising a protein having heparanase (endo- β -D-glucuronidase) catalytic activity or being cleavable so as to acquire said heparanase catalytic activity, said protein including a polypeptide at least 60% homologous to at least one of SEQ ID NOs: 10, 14, or 44 or portions thereof, the preparation being substantially free of a CXC chemokine or PAI1.

20. (Amended) An isolated protein having heparanase (endo- β -D-glucuronidase) catalytic activity or being cleavable so as to acquire said heparanase catalytic activity, said protein including a polypeptide at least 60% homologous to at least one of SEQ ID NOs: 10, 14, or 44 or portions thereof, said isolated protein being characterized by insect cell derived sugar [prosthetic groups] post-translational modifying groups.

22. (Amended) An isolated protein having heparanase catalytic (endo- β -D-glucuronidase) activity or being cleavable so as to acquire said heparanase catalytic activity, said protein including a polypeptide at least 60% homologous to at least one of

SEQ ID NOs: 10, 14, or 44 or portions thereof, said isolated protein being characterized by non-human cell derived sugar [prosthetic groups] post-translational modifying groups.

24. (Amended) A preparation comprising a protein of about 50 or about 65 kDa as determined by a denaturing polyacrylamide gel electrophoresis, said protein having heparanase (endo- β -D-glucuronidase) catalytic activity or being cleavable so as to acquire said heparanase catalytic activity, respectively, said protein including a polypeptide at least 60% homologous to at least one of SEQ ID NOs: 10, 14, or 44 or portions thereof, the preparation being free of non-heparanase polypeptides encoded by human nucleic acid sequences.

26. (Amended) An isolated protein of about 50 or about 65 kDa as determined by a denaturing polyacrylamide gel electrophoresis, said protein having heparanase (endo- β -D-glucuronidase) catalytic activity or being cleavable so as to acquire said heparanase catalytic activity, respectively, said protein including a polypeptide at least 60% homologous to at least one of SEQ ID NOs: 10, 14, or 44 or portions thereof, said isolated protein being substantially devoid of glycosilation.

28. (Amended) A preparation comprising a protein of about 50 or about 65 kDa as determined by a denaturing polyacrylamide gel electrophoresis, said protein having heparanase (endo- β -D-glucuronidase) catalytic activity or being cleavable so as to acquire said heparanase catalytic activity, respectively, said protein including a polypeptide at least 60% homologous to at least one of SEQ ID NOs: 10, 14, or 44 or portions thereof, the preparation being substantially free of a CXC chemokine or PAI1.

30. (Amended) An isolated protein of about 50 or about 65 kDa as determined by a denaturing polyacrylamide gel electrophoresis, said protein having heparanase (endo- β -D-glucuronidase) catalytic activity or being cleavable so as to acquire said heparanase catalytic activity, respectively, said protein including a polypeptide at least 60% homologous to at least one of SEQ ID NOs: 10, 14, or 44 or

portions thereof, said isolated protein being characterized by insect cell derived sugar [prosthetic groups] post-translational modifying groups.

32. (Amended) An isolated protein of about 50 or about 65 kDa as determined by a denaturing polyacrylamide gel electrophoresis, said protein having heparanase (endo- β -D-glucuronidase) catalytic activity or being cleavable so as to acquire said heparanase catalytic activity, respectively, said protein including a polypeptide at least 60% homologous to at least one of SEQ ID NOs: 10, 14, or 44 or portions thereof, said isolated protein being characterized by non-human cell derived sugar [prosthetic groups] post-translational modifying groups.

40. (Amended) An isolated protein at least 70 % homologous to SEQ ID NO:10, 14 or 44, the protein having heparanase (endo- β -D-glucuronidase) catalytic activity or being cleavable so as to acquire said heparanase catalytic activity, said isolated protein being characterized by insect cell derived sugar [prosthetic groups] post-translational modifying groups.

44. (Amended) A preparation comprising a protein having a pair of glutamic acid [s] residues participating in its active site and having heparanase (endo- β -D-glucuronidase) catalytic activity or being cleavable so as to acquire said heparanase catalytic activity, said protein including a polypeptide at least 60% homologous to at least one of SEQ ID NOs: 10, 14, or 44 or portions thereof, the preparation being free of non-heparanase polypeptides encoded by human nucleic acid sequences.

46. (Amended) An isolated protein having a pair of glutamic acid [s] residues participating in its active site and having heparanase (endo- β -D-glucuronidase) catalytic activity or being cleavable so as to acquire said heparanase catalytic activity, said protein including a polypeptide at least 60% homologous to at least one of SEQ ID NOs: 10, 14, or 44 or portions thereof, said isolated protein being substantially devoid of glycosilation.

48. (Amended) A preparation comprising a protein having a pair of glutamic acid [s] residues participating in its active site and having heparanase (endo- β -D-glucuronidase) catalytic activity or being cleavable so as to acquire said heparanase catalytic activity, said protein including a polypeptide at least 60% homologous to at least one of SEQ ID NOs: 10, 14, or 44 or portions thereof, the preparation being substantially free of a CXC chemokine or PAI1.

50. (Amended) An isolated protein having a pair of glutamic acid [s] residues participating in its active site and heparanase (endo- β -D-glucuronidase) catalytic activity or being cleavable so as to acquire said heparanase catalytic activity, said protein including a polypeptide at least 60% homologous to at least one of SEQ ID NOs: 10, 14, or 44 or portions thereof, said isolated protein being characterized by insect cell derived sugar [prosthetic groups] post-translational modifying groups.

52. (Amended) An isolated protein having a pair of glutamic acid [s] residues participating in its active site and having heparanase catalytic (endo- β -D-glucuronidase) activity or being cleavable so as to acquire said heparanase catalytic activity, said protein including a polypeptide at least 60% homologous to at least one of SEQ ID NOs: 10, 14, or 44 or portions thereof, said isolated protein being characterized by non-human cell derived sugar [prosthetic groups] post-translational modifying groups.

54. (Amended) A preparation comprising a protein having heparanase (endo- β -D-glucuronidase) catalytic activity or being cleavable so as to acquire said heparanase catalytic activity, said protein being capable of eliciting an anti-heparanase antibody, said protein including a polypeptide at least 60% homologous to at least one of SEQ ID NOs: 10, 14, or 44 or portions thereof, the preparation being free of non-heparanase polypeptides encoded by human nucleic acid sequences.

56. (Amended) An isolated protein having heparanase (endo- β -D-glucuronidase) catalytic activity or being cleavable so as to acquire said heparanase

catalytic activity, said protein being capable of eliciting an anti-heparanase antibody, said protein including a polypeptide at least 60% homologous to at least one of SEQ ID NOs: 10, 14, or 44 or portions thereof, said isolated protein being substantially devoid of glycosilation.

58. (Amended) A preparation comprising a protein having heparanase (endo- β -D-glucuronidase) catalytic activity or being cleavable so as to acquire said heparanase catalytic activity, said protein being capable of eliciting an anti-heparanase antibody, said protein including a polypeptide at least 60% homologous to at least one of SEQ ID NOs: 10, 14, or 44 or portions thereof, the preparation being substantially free of a CXC chemokine or PAI1.

60. (Amended) An isolated protein having heparanase (endo- β -D-glucuronidase) catalytic activity or being cleavable so as to acquire said heparanase catalytic activity, said protein being capable of eliciting an anti-heparanase antibody, said protein including a polypeptide at least 60% homologous to at least one of SEQ ID NOs: 10, 14, or 44 or portions thereof, said isolated protein being characterized by insect cell derived sugar [prosthetic groups] post-translational modifying groups.

62. (Amended) An isolated protein having heparanase catalytic (endo- β -D-glucuronidase) activity or being cleavable so as to acquire said heparanase catalytic activity, said protein being capable of eliciting an anti-heparanase antibody, said protein including a polypeptide at least 60% homologous to at least one of SEQ ID NOs: 10, 14, or 44 or portions thereof, said isolated protein being characterized by non-human cell derived sugar [prosthetic groups] post-translational modifying groups.

64. (Amended) An isolated protein having heparanase catalytic (endo- β -D-glucuronidase) activity or being cleavable so as to acquire said heparanase catalytic activity, said protein including a polypeptide at least 60% homologous to at least one of SEQ ID NOs: 10, 14, or 44 or portions thereof, said protein being capable of eliciting an anti-heparanase antibody.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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Commissioner of Patents and Trademarks
Washington, D.C. 20231
USA

AFFIDAVIT UNDER 37 CFR 1.132

I, Iris Pecker, am the Head of Department of Insight Ltd. at Petach Tikva, Israel. I have a PhD in Molecular Biology, and received my degree from The Hebrew University of Jerusalem. My professional specialization is in gene and protein discovery and development.

I am an inventor of the present invention. I have read the present application and the new and amended claims, as well as the Office Action from the Examiner and the accompanying references. In support of the accompanying Response to this Office Action, I set forth below the results of experiments which were performed in my laboratory.

Briefly, the present invention relates to isolated heparanase proteins, which are preferably recombinant, and which are optionally and more preferably at least 60% homologous to the polypeptide sequences disclosed in the present Application. The heparanase protein of the present invention has many significant uses. It may also be

produced in many different expression systems, such as insect cell expression systems for example.

The present invention represents a significant, non-obvious, inventive advance over the prior art such as Fuks (US Patent No. 5,362,641), which failed to identify a sequence for heparanase, or Hoogwerf (PCT Application No. WO 95/04158), which failed to correctly identify a heparanase sequence. In fact, these two references illustrate many of the pitfalls for an inventor who wished to determine the amino acid sequence of heparanase at the time of filing the present application.

Although the description of the sequence of events which led to the determination of the amino acid sequence of heparanase appears to be quite straightforward, it does not include all of the many problems and obstacles that were overcome in order to clone heparanase. Heparanase was an unusually difficult protein to sequence, largely because identification of true heparanase was so problematic. Hoogwerf was one of many inventors who thought that they had obtained the true heparanase sequence, only to discover that a contaminant present in the "pure heparanase" had been sequenced instead. In the case of Hoogwerf, the sequenced contaminant turned out to be the CXC chemokine CTAP III, a low molecular weight chemokine which has no homology to heparanase, as described above. However, it could just as easily have been one of the many other contaminants which have been mistakenly identified as heparanase, such as PAI1, the protein against which Fuks mistakenly raised an "anti-heparanase" antibody. As described above, the antibody of Fuks was later determined to bind to PAI1 rather than heparanase. PAI1 has no heparanase activity, but is present in a significant amount in the protein mixture obtained after the purification procedure of Fuks.

Many different processes for purifying heparanase have been proposed, such as that of Fuks, yet all have a common result: production of a mixture with many significant protein components, only one of which is heparanase. Like Fuks, many different scientists have mistakenly identified various proteins as being heparanase; a partial list is provided in the attached Appendix B, which provides a listing of only a sample of some of the many different proteins which were thought to be "pure" heparanase. Like Fuks, such a mistaken identification resulted at least in part from the belief that the particular purification process followed actually produced a pure heparanase.

I, as one of the inventors, also found that the purification process which we followed did not produce pure heparanase, but rather produced a mixture of proteins. Unlike previous scientists, however, we were able to overcome such a lack of purity by following a complex and difficult path to clone heparanase.

The purification process which we performed followed the teachings of Fuks, resulting in a mixture of proteins after the performance of Mono-S HPLC purification (col 16, lines 46-54 of Fuks). This mixture of proteins is described in the attached Appendix A. The mixture had heparanase activity, as in fact a portion of this mixture included heparanase itself. However, it also included other proteins, including PAI-1, a known contaminant of heparanase obtained through protein purification.

The performance of tryptic digestion and microsequencing of the mixture of proteins resulted in multiple protein (amino acid) sequences being obtained. We compared these sequences against the database of known proteins, and found that a number of such sequences did not match any known protein. These sequences are given in Appendix C, with some exemplary identified sequences from known proteins. The presence of multiple unidentified sequences was a concern to us, as we knew that many previous "heparanase" sequences or purified proteins had actually proven to be a contaminant of a purified heparanase, rather than heparanase itself.

We were able to select the correct short sequence obtained from microsequencing by using the database to identify a single clone. The sequence of this clone was used to isolate an extended cDNA and the clones were then assembled to form the correct sequence. However, highly surprisingly, a polypeptide was obtained which had 543 amino acids and a calculated molecular weight of 61,178 daltons. Such a finding was surprising because Fuks taught that the *expected* molecular weight of heparanase was 50 kD (col 15, lines 19-23 and 50-55).

We then decided to produce heparanase *(in cell culture)* from the cloned gene, in order to examine the activity of the gene product. as demonstrated in Appendix D. Attempting to transfect a mammalian cell line was not possible, because it would result in the problem of being unable to distinguish heparanase activity produced by the product of the transfected heparanase gene, and that of native heparanase, since the most commonly used mammalian cell lines have basal endogenous heparanase activity, as seen for

example in the cell lines 293 and CHO (Chinese hamster ovary cells) . Indeed, as we later demonstrated had we transfected the cloned gene into bacteria which is the most obvious expression system or yeast, no activity would have been observed and we would have assumed that we had not obtained the true heparanase gene.

Bacteria, yeast and mammalian cell lines are standard expression systems for mammalian proteins such as heparanase. Failure to obtain active heparanase in these systems is due to the lack of correct post translational modifications. Other cell expression systems, apart from mammalian cells, are usually not expected to be able to overcome this problem. These expression systems may also fail to produce a protein with the correct and apparently essential post-translational modifications, since such modifications differ between different cell systems. Only by choosing an unusual cell expression system, and transfecting insect cells with the heparanase gene sequence, using the Baculovirus expression system, were we able to demonstrate heparanase activity encoded by the isolated cDNA. Insect cells are not regularly used for mammalian proteins, and were certainly not the cell line of choice for the expression of a newly identified gene. Appendix E demonstrates why insect cells are not usually a cell line of choice for mammalian proteins, and also some of the typical uses for insect cells with the Baculovirus expression system for gene transfection.

As described in the present Application (page 78), the use of insect cells with the Baculovirus expression system results in the production of active heparanase gene product. However, the full length polypeptide for which the heparanase gene codes, which is about 66 kDa, is actually a prepro-heparanase form of the protein, and has no heparanase activity. Instead, this form of the protein is cleavable to form a protein having heparanase activity.

A residual level of such activity can be detected due to the effect of non specific protease activity, which results in the activation of a minor fraction of the recombinant enzyme. We found that the detection of heparanase activity required several micrograms of recombinant enzyme, a quantity that is much higher than expected from a catalytic enzyme. This finding raised doubts about the functional identification of the cloned cDNA, and caused us to doubt whether the correct gene had been identified and cloned.

Although cleavage of the prepro-heparanase results in catalytic activity of the enzyme, attempts to obtain an actual heparanase protein through protein cleavage is likely to result in inactive protein. The activating protease is unknown and using non-specific proteases such as trypsin requires extensive studies in order to obtain an activated protein rather than a degraded protein. The pro-heparanase protein can be divided into three sections: an 8 kDa section, a 6 kDa section and the main 45 kDa section. In order to produce heparanase, the 6 kDa section is removed and the 8 kDa section joins to the 45 kDa section.

Thus, we were forced to overcome many barriers in order to obtain the sequence of heparanase, which was an unusually difficult protein to sequence even at the time of filing of the present application.

I hereby certify that the above facts and statements are true and complete, to the best of my knowledge.

Ira Pecker

**Dr. Iris Pecker**

**Date:** 21/4/03

Appendix A

The proteins identified by the inventors following the performance of Mono-S HPLC purification were found to be as follows: PAI-I, Nexin-I, Vimentin, Grp94/endoplasmin, FLT receptor, Tryptase.

Appendix B

The following is a list of only a sample of some of the many different proteins which were once erroneously thought to be "pure" heparanase:

1. PAI-1 – antibodies generated against purified heparanase by Fuks & Vlodavsky (inventors of US Patent No. 5,362,641) were found to detect PAI-1 rather than heparanase (Vlodavsky, personal)

2. CTAPIII -Hoogewerf et al. J Biol Chem 1995 Feb 17;270(7):3268-77

3. GRP94/endoplasmin (De Vouge et al. Int J Cancer 1994 Jan 15;56(2):286-94)

Appendix C

Peptides identified as *nexin*-I: (Vlodavsky, personal)

TFVAADGK

SENLHVSHILQK

SYQVPMLAQLSVFR

XGSTSAPNDLXYNFIE(?)XPY

LVLVNAVYFK

HNPTGAVLFM?XQI

Unindentified peptides: (Vlodavsky, personal)

XYGPDVGQPR

QVFFEAG?NYH?LVDENE

GLSPAYLR

XATDED(Y/L)(T/L)N(P/A)DV

VAASIYT

S?VQLF?(S/G)N(T/K)

SFLK

LLR

(not listed: sequences for peptides of the other identified proteins)

Appendix D

Expression of heparanase in E.coli, which is the easiest expression system and which would be the obvious choice for a person skilled in the art, results in unfolded or misfolded polypeptide which appears in inclusion bodies and which is obviously inactive. Yeast cannot produce active heparanase because no processing of pro-heparanase occurs in any microbial system, including that of yeast. Expression of full length heparanase cDNA in yeast results in secretion of a latent preproheparanase, which has no measurable activity (see for example US Patent No. 6348344: Genetically modified cells and methods for expressing recombinant heparanase and methods of purifying same).

Appendix E

Insect cells are not a typical choice for protein expression, since post-translational processing for mammalian proteins is typically not performed properly in insect cells. For the present invention, heparanase activity was detected due to a minor proteolytic activity of an endogenous insect cell non-specific protease. This resulted in a small fraction of partially processed active heparanase which could be detected due to the use of the most sensitive heparanase ECM assay. However, as described above, such a higher level of activity would be expected from a recombinant enzyme.

Appendix F

It should be noted that the exact, complete structure of heparanase is not currently known. It has been shown that mature active heparanase contains two subunits 45kDa and 8 kDa. The interaction is non-covalent and does not involve S-S bridges. There is no information regarding the location or chronology of processing events, although it has been shown that these two subunits are separated by another peptide in the pre-pro form of heparanase, in which the two subunits and the third peptide together form a single long peptide.